 doi:10.20944/preprints202012.0679.v1

PREVALENCE OF ANTIMICROBIAL RESISTANCE (AMR) *SALMONELLA* SPP. AND *ESCHERICHIA COLI* ISOLATED FROM BROILERS IN EAST COAST MALAYSIA

Shamsaldeen Ibrahim[1,2], Loh Wei Hoong[3], Yip Lai Siong[3] Zaharuddin Mustapha[4], Che Wan Salma Che Wan Zalati[1], Erkihun Aklilu[1], Maizan Mohamad[1], Nor Fadhilah Kamaruzzaman[1*]

[1]Faculty of Veterinary Medicine, University Malaysia Kelantan, 16100 Pengkalan Chepa, Kelantan, Malaysia

[2]Faculty of Veterinary Science, University of Nyala, PO Box 155 | South Darfur State, Nyala, Mousseh district, south of the city, Sudan.

[3]Rhone Ma Malaysia Sdn. Bhd., Lot 18A & 18B, Jalan 241, Seksyen 51a , 46100 Petaling Jaya, Selangor

[4]Department of Veterinary Services Pahang, Jalan Sri Kemunting 2, 25100 Kuantan, Pahang.

Corresponding author: norfadhilah@umk.edu.my, +6097717330

 doi:10.20944/preprints202012.0679.v1

Abstract:

Salmonella species (spp) and *Escherichia coli (E. coli*) are the most common infectious pathogens in poultry. Antimicrobials were given either for the treatment or growth promoters that can increase the possibility of emergence of bacterial resistance towards antimicrobials**.** The aim of this study was to determine the prevalence of antimicrobial resistant (AMR) *Salmonella* spp and *E. coli* isolated from a sample of broiler farms in East Coast Malaysia from 2018-2019. A total of 384 cloacal swabs were collected from broilers farms in Kelantan, Terengganu, and Pahang. The bacteria were isolated and confirmed by bacteriological and serological methods. Following that, confirmed isolates were subjected to antimicrobial susceptibility test. *Salmonella* spp and *E. coli* were recovered from the cloacal swabs samples with the overall prevalence of 6.5% and 51.8% respectively. In Kelantan, Terengganu and Pahang, the prevalence of *Salmonella* spp were 7%, 6.5% and 5.8% respectively, while the prevalence for *E. coli* were 50%, 48.3% and 58% respectively. *Salmonella* spp and *E. coli* displayed resistance towards the following antimicrobials: erythromycin (100% for both pathogens), chloramphenicol (76.2%, 84.5%), tetracycline (62%, 94.6%), ampicillin (47.7%, 87%), sulfamethoxazole/trimethoprim (42.9%, 83.3%), ciprofloxacin (4.8%, 23.8%), nalidixic acid (9.6%, 60.7%), streptomycin (19%,66%), and kanamycin (28.6%,57%), cephalotin (0%, 11%), gentamicin (0%, 20.2%) respectively. No resistance were recorded towards colistin for both pathogens. Multidrug resistance (MDR) was recorded in 82% of *Salmonella* spp and 100% of *E. coli.* These findings demonstrate the high prevalence of MDR *Salmonella* spp. and *E. coli* in broiler farms in East coast Malaysia. This could be attributed to the excessive use of antimicrobial agents by the poultry farm owners. Enhanced control measures and a strong monitoring system should be urgently implemented to reduce the emergence of antimicrobial resistance that is harmful to public health.

Keywords; Antimicrobial resistance, *Salmonella* species, *E.coli*, Broiler chickens, Malaysia

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 December 2020 doi:10.20944/preprints202012.0679.v1

INTRODUCTION

The poultry industry is the primary agricultural sector in Malaysia which contributes 62.9% from the total GDP in the animal farming industry (Amna *et al*., 2020). It has transformed with increasing in the number of production as well as increasing number of integrators. Malaysians consume approximately 1.8 million chicken and 2.8 million eggs daily, which is translated for annual consumption of 31 kg of meat and 16.6kg of eggs per capita. This consumption is considered among the highest in the world, due to the large Muslim population and higher price of other protein sources such as beef and mutton (Orissa International Sdn Bhd, 2017). In Peninsular Malaysia, the supply is by approximately 3,200 broiler farms which includes contract and independent farmers, as well as large vertically integrated farms. Though the industry is expanding, the sector still faces many challenges, which include infectious disease outbreak of avian salmonellosis and colibacillosis (Chuah et al., 2018; Daud et al., 2014).

Avian salmonellosis can be caused by intestinal colonization and invasion by *Salmonella* serovars resulting in enteritis, septicemia, and mortality in animal. Some Salmonella serovars, particularly *Salmonella* Typhimurium and *Salmonella* Enteritidis, can persist in the digestive tracts of chickens (Huang et al., 2009). *Salmonellosis* is caused by non-typhoidal *Salmonella* and typically characterised by gastroenteritis syndrome in human (Antunes et al., 2016). Avian colibacillosis is a significant infectious disease caused by pathogenic *E. coli* strains and causes massive economic losses to the poultry industry due to high morbidity, mortality, and cost of treatment and prevention (Kim et al., 2020). The condition is characterised by respiratory infection, yolk sac infection, coli granuloma, enteritis, cellulitis omphalitis, swollen head syndrome, septicemia, polyserositis, and salpingitis (Kabir, 2010). Transmission of *Salmonella* and *E. coli* to humans could occur through the consumption of contaminated poultry and handling of the raw poultry (Chuah et al., 2018).

Antimicrobials are used widely for treatment, prevention of the infectious disease in livestock, and excessive use and misuse of antimicrobials, in part were associated with increasing rates of antimicrobial resistance among pathogens isolated from animal . There is growing concerned that widespread antimicrobial use has led to the

 doi:10.20944/preprints202012.0679.v1

emergence of some organisms resistant to most or all antimicrobials (Teillant & Laxminarayan, 2015). Antimicrobial resistance present in bacteria from production animals may lead to therapy failure and economic losses for the farmer, and transferring of resistance to potential human pathogenic bacteria, and likewise causing treatment difficulties (van den Bogaard & Stobberingh, 1999). Antimicrobial resistance is a big challenge to the Malaysian public health. Increasing cases of treatment failure in human and animal were reported in recent years showed that the pathogen do not respond to the antimicrobials administered for the treatment (Alreshidi, M.A et al., 2018).

Despite the data available for the antimicrobial resistance in pathogen isolated in poultry in Malaysia, we find very limited data on the prevalence of antimicrobial resistance *Salmonella* spp and *E. coli* in the poultry farm in East Coast Malaysia; Kelantan, Terengganu and Pahang (Jajere et al., 2019). Thus the aim of the present study was to determine the prevalence of AMR *Salmonella* spp and *E.coli* isolated from broiler farms in these three states.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 December 2020 doi:10.20944/preprints202012.0679.v1

MATERIALS AND METHODS

Ethical statement

The current study was conducted at Zoonotic laboratory, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan. The study protocols, procedures, and consent form were approved by the Institutional Animal Care and Use Committee of Universiti Malaysia Kelantan (UMK/FPV/ACUE/PG/2/2019).

Sample size determination and sampling method

The sample size was determined by using StatCalc from Epi-Info (7) using a formula based on Thrusfield (2007). The calculation was based on the assumption of an expected prevalence of 50% for *E.coli* based on the previous literature (Jamnah *et al*., 2014).

$$n = \frac{1.962\ Pexp\ (\ 1 - Pexp)}{d\ 2}$$

Where, n=required sample size 1.96= 95% level of confidence

P exp =exposed prevalence d=desired absolute decision

Cloacal swabs were obtained from broilers from 30 different farms in Kelantan, Terengganu, and Pahang. Farms were selected based on the list of broiler farms provided by the Department of Veterinary Services, Malaysia. Farm selection were performed by the multistage random selection method. Cloacal swabs were collected aseptically using sterile swabs with Ames transport media. Following sample collection, the samples were immediately transported back to the laboratory under cold storage for further processing.

Isolation and identification of bacteria

Prior to bacteria isolation, pre-enrichment was performed by inoculating the swabs into buffered peptone water (BPW; Oxoid, UK) followed by incubation at 37°C for 24 hours. For *E. coli* isolation, the enriched BPW was streaked on MacConkey agar

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 December 2020 doi:10.20944/preprints202012.0679.v1

plates (MAC; Oxoid, UK) and incubated aerobically for 24 hours at 37°C. Suspected lactose fermentative *E. coli* colonies were sub-cultured on eosin methylene blue agar (EMB; oxoid, UK) for another 24 hours at 37°C. Suspected *E.coli* which displayed green metallic shine colonies were further subjected to biochemical testing. Colonies that exhibited acid slant, acid butt, and no H_2S production on triple sugar iron, indole and decarboxylase positive, regardless of motility, were considered to be *E. coli* and were subcultured and stored in glycerol stock and kept at -80°C until ready to be used.

For *Salmonella* isolation, 0.1 mL of BPW mixture were inoculated in Rappaport – Vassiliadis Soya Pepton Broth (RVS; Oxoid, UK) at 42°C for 24 hours for selective enrichment. Following that, the RVS mixture was streaked in xylose-lysine-desoxycholate agar (XLD; Oxoid, UK) and incubated aerobically for 24 hours at 37°C. After 24 hrs, the plate was examined for the presence of suspected *Salmonella* spp. The suspected colonies were subjected to biochemical test followed by latex agglutination test using the commercial available polyvalent antisera (Oxoid *Salmonella* test kit DR1108A) to screen for *Salmonella* flagellar antigen. Briefly, a loop full of suspected colonies were emulsified with one drop of 0.85% sodium chloride on the reaction card to produce the smooth suspension. Then a drop of *Salmonella* latex reagent was added and mixed with the organism suspension with the clean mixing stick. *Salmonella* isolates will cause an agglutination in the reaction.

Antimicrobial susceptibility test

The antimicrobial susceptibility for all isolates were determined through the standard antimicrobial disk diffusion test protocol by the Clinical and Laboratory Standard Institute (CLSI, 2016). The following antimicrobial commercial disc from Oxoid, UK were used in this study: tetracycline(TE; 30 µg), chloramphenicol (C; 30 µg), ampicillin (AMP; 10 µg), cephalothin (CL; 30µg), streptomycin (S; 10 µg), gentamicin (CN; 10 µg), sulfamethoxazole/trimethoprim (SXT; 25 µg), nalidixic acid (NA;30 µg), ciprofloxacin (5 µg), erythromycin(E; 15 µg), kanamycin (K; 30 µg) and Clostine sulphate (CT; 10 µg). All selected antimicrobials are commonly used for the treatment of infections associated with *E. coli*, and *Salmonella* based on the recommendation by World Organisation for Animal Health (OIE, 2014). Briefly, 0.5 McFarland bacterial suspension was prepared and plated on the agar surface. Six

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 December 2020 doi:10.20944/preprints202012.0679.v1

paper discs were placed onto each agar plate using a dispenser. The plate was incubated at 37°C for 18 hours. The resulting zones of inhibition (ZOI) was measured in millimetre using a vernier caliper and measurements were rounded off to the nearest whole number. The antimicrobial sensitivity profiles of the isolates were determined following the zone of inihibition diameter breakpoints and interpretative categories (susceptible, intermediate or resistant) for Enterobacteriaceae as recommended by CLSI (CLSI,2016) (Table 1).

Determination of multiple antimicrobial resistance (MAR) indexes

MAR was calculated as reported by Christopher and Ali (2013) as follows:

$$\text{MAR index} = \frac{\text{Number of antimicrobials to which the isolate showed resistance}}{\textit{Number of total antibiotics exposed to the isolate}}$$

Results were interpreted according to the criteria of Nandi & Mandal: MAR index ≤ 0.2 was considered low risk, while ≥ 0.2 indicated a high risk of antimicrobial contamination (Akande et al., 2019)

Statistical analysis

The results were analyzed statistically using the Graph Pad Prism version 8. The level of significance was determined at 95% confidence level and $p<0.05$.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 December 2020 doi:10.20944/preprints202012.0679.v1

RESULTS

Prevalence of *Salmonella* spp and *E. coli* in broiler poultry farms in Kelantan, Terengganu and Pahang.

Of 384 samples, a total of 25 *Salmonella* spp. and 199 *E. coli* were isolated with the overall prevalence of 6.6% and 51.8%, respectively. In Kelantan, Terengganu and Pahang, the prevalence of *Salmonella* spp were 7%, 6.5% and 5.8% respectively, while the prevalence for *E. coli* were 50%, 48.3% and 58% respectively. Table 2 summarizes the results for the prevalence and distribution of *Salmonella* spp. and *E. coli* isolated from broilers in the three state.

Salmonella and *E. coli* susceptibility towards antimicrobial tested

Overall Salmonella and E. coli susceptibility towards antimicrobial tested

To determine *Salmonella* spp. and *E. coli* isolates susceptibility towards selected antimicrobials, antimicrobial susceptibility test were performed using disc diffusion methods. *Salmonella* spp and *E. coli* displayed resistance towards the following antimicrobials; erythromycin (100% for both pathogen), chloramphenicol (76.2%, 84.5%), tetracycline (62%, 94.6%), ampicillin (47.7%, 87%), sulfamethoxazole/trimethoprim (42.9%, 83.3%), ciprofloxacin (4.8%, 23.8%), nalidixic acid (9.6%, 60.7%), streptomycin (19%,66%), and kanamycin (28.6%,57%), cephalotin (0%, 11%), gentamicin (0%, 20.2%) respectively. All *Salmonella* and *E. coli* isolates were sensitive towards colistin antimicrobial. Table 3 summarises *Salmonella* and *E. coli* towards all antimicrobials tested.

Distribution of antimicrobial resistance in Kelantan, Terengganu and Pahang

In Kelantan, >50% *Salmonella* spp recorded resistance towards tetracycline, chloramphenicol, ampicillin and erythromycin. While >50% *E. coli* recorded resistance towards all antimicrobials tested except for ciprofloxacin, cephalothin and colistin sulphate.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 December 2020 doi:10.20944/preprints202012.0679.v1

In Terengganu >50% *Salmonella* spp recorded resistance towards tetracycline, chloramphenicol, sulfamethoxacole/trimethophrim and erythromycin. While >50% *E. coli* isolates demonstrated resistance towards almost all antimicrobials except cephalotin and colistin sulphate.

Finally in Pahang, >50% *Salmonella* spp recorded resistance towards tetracycline, chloramphenicol, ampicillin and erythromycin. While >50% *E. coli* isolates demonstrated resistance towards almost all antimicrobials except cephalotin and colistin. In summary, the highest resistance for *Salmonella and E. coli* for all three states were towards tetracycline, chloramphenicol and erythromycin. Table 4 summarised the distribution of AMR in Kelantan, Terengganu and Pahang.

Salmonella spp and _E. coli_ multi drug resistance (MDR) profile

The MDR profile for *Salmonella* spp and *E. coli* were also tabulated. A total of 81% of *Salmonella* spp isolates showed multidrug resistance profile (resistance to >1 antimicrobial). This includes 4.8% isolates that were resistance to six, seven and eight antimicrobials, 14.2% to five antimicrobials, 42.8% to four antimicrobials and 9.5% to three antimicrobials, respectively. The most predominant MDR profile for *Salmonella* spp. were TE-C-AMP–E, TE-C-SXT–E, C-AMP-K–E and C-AMP-SXT–E.

In parallel, 5.9% of *E. coli* were resistant to ten antimicrobials, 10.7% to nine antimicrobials, 21.4% to eight antimicrobials, 20.2% to seven antimicrobials, 17.2% to six antimicrobials, 12.5% to five antimicrobials, 6.5% to four antimicrobials and 3.5% to three antimicrobials, respectively. MDR profile *E. coli* isolates showed varieties of AMR profile, where 56 different MDR profile were recorded. The most predominant antibiotype were TE-C-AMP-S-SXT-NA-K-E, TE-C-AMP-S-SXT-NA-E and TE-C-SXT-NA-E-CIP. Table 4 summarises the MDR profile for *Salmonella* spp and *E. coli*.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 December 2020 doi:10.20944/preprints202012.0679.v1

Multiple Antimicrobial Resistance (MAR) Index

Multiple antimicrobial resistance index is helpful in analyzing health risk, as well as to check the extent of antimicrobial resistance. The MAR index was calculated for both *Salmonella* and *E. coli* isolates. The analysis showed 71% of *Salmonella* isolates have MAR > 0.2, while 96% *E. coli* isolates showed MAR index > 0.2 (Table 5), suggested that the isolates originated from the high-risk source of contamination where the antimicrobials are commonly used.

DISCUSSIONS

Increasing AMR cases in human, in part, has been correlated with transmission of the pathogen from animal to human. Here we found that *Salmonella* and *E. coli* isolated from broilers in East coast Malaysia displayed multi drug resistance towards commonly used antimicrobials used in animal. We also find that majority of the isolated *Salmonella* and *E. coli* has MAR index> 0.2.

Salmonella spp and *E. coli* are the predominant bacteria associated with bacterial infection in poultry. These organisms are known to result in serious problems to poultry health leading to mortality, reduced production and increased expense in the cost of prevention and treatment of the disease. Broad diversity of antimicrobials are used to raise poultry in most countries, mostly through the oral route, to prevent and to treat disease, but also to enhance growth and productivity (Nhung et al., 2017). The findings of our study is in agreement with a study conducted in Selangor, Malaysia that reported a high prevalence rate of *E. coli* (60%) compared to only 7.5% of *Salmonella* spp. isolated from the same samples (Geidam, 2012). Another study reported the prevalence of *Salmonella* isolated from village chicken in Malaysia was 2.5% (Jajere et al., 2019). The low prevalence of *Salmonella* isolated from poultry was also reported in other countries such as Nigeria (2%) and European countries (Gonçalves-Tenório et al., 2018; Chinasa. et al., 2019). It is interesting to note that the same trend does not appear to be so in Bangladesh as a study showed a high prevalence (48%) of *Salmonella* isolated from poultry (Islam et al., 2017).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 December 2020 doi:10.20944/preprints202012.0679.v1

Antimicrobial resistance in chickens is a common problem in Malaysia and other developing countries due to the practice antimicrobials used as feed additives and prophylactic treatment of infectious diseases. Our study found that 100% *Salmonella* and *E. coli* resistance towards erythromycin antimicrobial, and this finding is in agreement with another study conducted in Bangladesh that reports the same trend of resistance (Islam et al., 2017). Our study also found high prevalence of multidrug resistance *Salmonella* and *E. coli* isolates which are in agreement by previous study conducted in Malaysia (Geidam, 2012). These findings provide evidence of the emergence of antimicrobials resistance of *Salmonella* spp and *E. coli* among poultry farm in Malaysia. It is interesting to note that all *Salmonella* and *E. coli* isolates were susceptible to colistin, though a recent study conducted in the same region detected *MCR-1 gene* which encoded colistin resistant in *E. coli* isolated in raw chicken meat in Kelantan, Malaysia (Aklilu & Raman, 2020). It is important to note that the study conducted by Aklilu and Raman were using molecular biology method that are known to be more sensitive compared to the disc diffusion method.

In conclusion, this finding indicated the high prevalence of multi- drugs resistant *Salmonella* spp. and *E.coli* in poultry farms in East Coast Malaysia and this, in part, could be attributed to the excessive use of antimicrobial agents by poultry farm owner and these potentially harmful to public health. Control measures and strong monitoring system should be urgently advocated and implemented in Malaysia to reduce the emergence of AMR. Also, further research on alternative to antimicrobials, good animal husbandry practice and biosecurity should be encouraged to replace the application of existing antimicrobials in animal health.

ACKNOWLEDGMENT

This work was supported by Rhone Ma Malaysia Sdn. Bhd grant number R/RMA/A06.00/00686A/002/2018/000541.The author express their appreciation to FPV, UMK Kelantan and Department Veterinary services, Malaysia for facilities to conduct this work. The author also would like to acknowledge the broiler farms owner for their assistance during the sampling procedures

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 December 2020 doi:10.20944/preprints202012.0679.v1

AUTHOR CONTRIBUTIONS

S. I. S. performed the experiment and wrote the manuscript. LWH, YLS· ZM helped during the sampling process, CWSCWZ helped during the bacterial isolation, EA, MM and NFK supervise the work and wrote the manuscript. We thank Dr. Raymond Choo for helping in proofreading of the manuscript.

CONFLICT OF INTEREST

No conflict of interest

 doi:10.20944/preprints202012.0679.v1

REFERENCES

1. Akande, E., Abodunrin, T., Oladejo, B., & Oladunmoye, M. (2019). Antibiogram and plasmid profiling of resistance bacteria isolated from the blood of Hepatitis C Virus positive individuals. *Journal of Microbiology & Experimentation*, *7*(3), 156–165.
2. Aklilu, E., & Raman, K. (2020). MCR-1 Gene Encoded Colistin-Resistant Escherichia coli in Raw Chicken Meat and Bean Sprouts in Malaysia. *International Journal of Microbiology*. https://doi.org/10.1155/2020/8853582
3. Alreshidi, M.A., Neela, V, Alsharari, A.S., Hamat, R.A., Alsalamah, A.A., Atshan, S.S., Alzoghaibi, O., and Shamsudin, M. N. (2017). Molecular typing and antibiotic resistance patterns of methicillin-resistant Staphylococcus aureus isolates from clinical samples in Malaysia : An update. *Tropical Biomedicine*, *34*(1), 1–6.
4. Amna, N., Mohammad, A., & Rosali, M. H. (2020). *The Development and Future Direction of Malaysia ' s Livestock Industry*. 1–7. Retrieved from https://ap.fftc.org.tw
5. Antunes, P., Mourão, J., Campos, J., & Peixe, L. (2016). Salmonellosis: The role of poultry meat. *Clinical Microbiology and Infection*, *22*(2), 110–121.
6. Chuah, L. O., Shamila Syuhada, A. K., Mohamad Suhaimi, I., Farah Hanim, T., & Rusul, G. (2018). Data on antibiogram and resistance genes harboured by Salmonella strains and their Pulsed-field gel electrophoresis clusters. *Data in Brief*, *17*, 698–702.
7. Clinical, L. S. A. I. (2016). *Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Susceptibility Testing Supplement M100S*.
8. Daud, N., Hatin, N., Abba, Y., Paan, F., Kyaw, T., Khiang, A., Jesse, F., Mohammed, K., Adamu, L., & Tijjani, A. (2014). An Outbreak of Colibacillosis in a Broiler Farm. *Journal of Veterinary Advances*, *4*(7), 648.
9. Geidam, Y. (2012). High Prevalence of Multi-drug Resistant Bacteria in Selected Poultry Farms in. *Aisan Journal of Animal and Veterinary Advances*, *7*(9), 891–897.
10. Gonçalves-Tenório, A., Nunes Silva, B., Rodrigues, V., Cadavez, V., & Gonzales-Barron, U. (2018). Prevalence of pathogens in poultry meat: A meta-

analysis of European published surveys. *Foods*, *7*(5).

11. Huang, T.-M., Lin, T. L., & Wu, C. C. (2009). Antimicrobial Susceptibility and Resistance of Chicken Escherichia Coli, Salmonella spp., and Pasteurella Multocida Isolates. *Avian Diseases*, *53*(1), 89–93. https://doi.org/10.1637/8268-021608-reg.1
12. Islam, J., Ahmed, T., & Hasan, K. (2017). Isolation and identification of Salmonella spp. from broiler and their antibiogram study in Sylhet, Bangladesh. *Journal of Applied Biology & Biotechnology*, *4*(03), 46–51.
13. Jajere, S. M., Hassan, L., Aziz, S. A., Zakaria, Z., Abu, J., Nordin, F., & Faiz, N. M. (2019). Salmonella in native “ village ” chickens (Gallus domesticus): prevalence and risk factors from farms in South-Central Peninsular Malaysia. *Poultry Science*, *98*(5961), 5970.
14. Jamnah, O., Faizal, H., Chandrawathani, P., Premaalatha, B., Rozita, L., & Ramlan, M. (2014). *Malaysian journal of veterinary research eperythrozoonosis (mycoplasma sp .) In malaysian pangolin*. *5*(June), 65–68.
15. Jindal, H. M., Ramanathan, B., Le, C. F., Gudimella, R., Razali, R., Manikam, R., & Sekaran, S. D. (2018). Comparative genomic analysis of ten clinical Streptococcus pneumoniae collected from a Malaysian hospital reveal 31 new unique drug-resistant SNPs using whole genome sequencing. *Journal of Biomedical Science*, 1–14.
16. Kabir, S. M. L. (2010). *Avian Colibacillosis and Salmonellosis : A Closer Look at Epidemiology , Pathogenesis , Diagnosis , Control and Public Health Concerns*. 89–114.
17. Kim, Y. Bin, Yoon, M. Y., Ha, J. S., Seo, K. W., Noh, E. B., Son, S. H., & Lee, Y. J. (2020). Molecular characterization of avian pathogenic Escherichia coli from broiler chickens with colibacillosis. *Poultry Science*, *99*(2), 1088–1095.
18. Mkk, F., Rashid, S. S., Mhm, N., Zaidul, I. S. M., Baharudin, R., & Nor, A. (2018). A clinical update on Antibiotic Resistance Gram-negative bacteria in Malaysia- a review. *Journal of International Pharmaceutical Research*, *45*, 270–283.
19. Nhung, N. T., Chansiripornchai, N., & Carrique-Mas, J. J. (2017). Antimicrobial Resistance in Bacterial Poultry Pathogens: A Review. *Frontiers in Veterinary Science*, *4*(August), 1–17.
20. Orissa International Pte. Ltd. (2017, March). Poultry Sector in South East Asia. In *Iowa Economic Development Authority*. Retrieved from

 doi:10.20944/preprints202012.0679.v1

www.iowaeconomicdevelopment.com

21. Teillant, A., & Laxminarayan, R. (2015). Economics of Antibiotic Use in U . S . Swine and Poultry Production. *Choices*, *30*(1), 1–11.
22. Thrusfield, M. (2007). Sample size determination. In *Veterinary Epidemiology*.
23. Chinasa, V., Eleazar, E., Scholastica, N., & Ike, A. C. (2019). Isolation , Identification and Antibiogram of Salmonella from Cloacal Swabs of Isolation , Identification and Antibiogram of Salmonella from Cloacal Swabs of Free Range Poultry in Nsukka , Nigeria. *Journal of Advcances Microbiology*, *17*(1), 1–9.
24. van den Bogaard, A. E., & Stobberingh, E. E. (1999). Antibiotic Usage in Animals. *Drugs*, *58*(4), 589–607.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 December 2020 doi:10.20944/preprints202012.0679.v1

Table 1: Zone diameter interpretative breakpoints for the tested antimicrobials (CLSI,2016)

Antimicrobial	Disc code & content (µg)	Zone of inhibition (mm)		
		Sensitive	Intermediate	Resistant
Penicillin	10 units	≥29	-	≤28
Ampicillin	AMP (10)	14	12-14	11
Oxacillin	OX (1)	≥18	-	≤17
Tetracycline	TE (30)	≥19	15-18	≤14
Gentamicin	CN (10)	≥15	13-14	≤12
Erythromycin	E (15)	≥23	14-22	≤13
Chloramphenicol	C (30)	≥18	13-17	≤12
Ciprofloxacin	CIP (5)	≥21	16-20	≤20
Kanamycin	K (30)	≥18	14-17	≤13
Nalidixic acid	NA (3)	≥19	14-18	≤13
Sulfamethoxazole -trimethoprim	SXT (25)	16	11-15	10
Streptomycin	S (10)	21	15-20	14
Vancomycin	VA (30)	≥17	15-16	≤14
Cephalexin	CF (30)	≥18	15-17	≤14
Colistin sulphate	CT (10)	≥11	-	≤10

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 December 2020 doi:10.20944/preprints202012.0679.v1

Table 2. Prevalence and distribution of *Salmonella* spp. and *E. coli* isolated from broiler in Kelantan, Terengganu dan Pahang

State/ Locality	No. of collected samples	Prevalence of *Salmonella* spp	Prevalence of *E. coli*
Kelantan			
Machang	40	0%	50%
Bachok	40	15%	45%
Tumpat	40	12.5%	62.5%
Pasir Mas	40	7.5%	37.5%
Jeli	40	0%	55%
total	200	**7%**	**50%**
Terengganu			
Marang	30	0%	33.3%
Hulu Terengganu	30	13.3%	63.3%
total	60	6.5%	48.3%
Pahang			
Kuantan	32	0%	65.6%
Pekan	32	0%	59.4%
Maran	30	13.3%	33.3%
Temerloh	30	10%	66.6%
total	**120**	**5.8%**	**58.0%**
Overall	**384**	**6.5%**	**51.8%**

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 December 2020 doi:10.20944/preprints202012.0679.v1

Table 3. *Salmonella* spp and *E. coli* susceptibility towards all antimicrobials tested.

Antimicrobials	Susceptible (%)		Intermediate (%)		Resistant (%)	
	Salmonella	*E. coli*	*Salmonella*	*E. coli*	*Salmonella*	*E. coli*
Tetracycline	38	5.3	0	0	62	94.6
Chloramphenicol	23.8	14.8	0	0.5	76.2	84.5
Ampicillin	52.3	12	0	0.5	47.7	87.5
Streptomycin	76.1	31	4.7	3	19	66
Gentamicin	100	75.6	0	4.2	0	20.2
Sulfamethoxazole/ trimethoprim	57.1	16	0	0.5	42.9	83.3
Nalidixic acid	90.4	39.3	0	0	9.6	60.7
Kanamycin	71.4	43	0	0	28.6	57
Erythromycin	0	0	0	0	100	100
Ciprofloxacin	95.2	72	0	4.2	4.8	23.8
Cephalothin	100	87	0	2	0	11
Colistin sulphate	100	100	0	0	0	0

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 December 2020 doi:10.20944/preprints202012.0679.v1

Table 4. Distribution of *Salmonella* spp and *E. coli* resistance in Kelantan, Terengganu and Pahang

Antimicrobials (potency µg)	Resistant (%)					
	Kelantan		Terengganu		Pahang	
	Salmonella	*E. coli*	*Salmonella*	*E. coli*	*Salmonella*	*E. coli*
Tetracycline	57.1	90.7	75	100	71.4	89.3
Chloramphenicol	57.1	91.6	100	84	71.4	78.7
Ampicillin	50	81.4	0	92	71.4	84.8
Streptomycin	35.7	60.1	25	96	14.2	62.1
Gentamicin	0	3.7	0	84	0	15.1
Sulfamethoxazole/ trimethoprim	35.7	88.8	100	88	14.2	71.2
Nalidixic acid	14.2	61.6	25	72	0	63.6
Kanamycin	28.5	56.4	25	72	28.5	53
Erythromycin	100	100	100	100	100	100
Ciprofloxacin	0	13.8	0	52	14.2	25.7
Cephalothin	0	7.4	0	20	0	12.1
Colistin sulphate	0	0	0	0	0	0

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 December 2020 doi:10.20944/preprints202012.0679.v1

Table 5. Antimicrobial resistance patterns and multiple resistance index (MAR) in *Salmonella* spp. isolate

No of antimicrobials	*Salmonella*			*E. coli*		
	MDR profile	% of isolates	MAR index	MDR profile	% of isolates	MAR index
12	N/A	N/A	N/A	N/A	N/A	N/A
11	N/A	N/A	N/A	TE - C-AMP-S - CN- SXT- NA -K- E- CIP- CL	1.2	0.9
10	N/A	N/A	N/A	TE - C-AMP-S - CN- SXT- NA -K- E- CIP TE - C-S - CN- SXT- NA -K- E- CIP- CL TE - C-AMP-S - CN- NA -K- E- CIP- CL	5.9	0.8
9	N/A	N/A	N/A	TE - C-AMP-S - CN- SXT- NA -K- E TE - C-AMP-S -SXT- NA -K- E- CIP TE - C-AMP-S - SXT- K- E- CIP- CL TE - C-AMP-S - NA -K- E- CIP- CL C-AMP-S - CN- SXT- NA -K- E- CIP TE - C-AMP-S - CN- SXT- NA -E- CIP	10.7	0.7
8	TE- C- AMP - S- SXT - K - E- CIP	4.8	0.7	TE - C-AMP-S - CN- SXT- E- CL TE - C-AMP-S - SXT- NA -K- E TE - C-AMP- S- SXT- NA -K- E TE - C-AMP-S - CN- SXT- K- E TE - C-AMP-S - CN- SXT- K- E TE - C-AMP-S - NA -K- E- CIP TE - C-AMP-S - CN- NA -K- E TE - AMP-S - CN- SXT- NA -K- E	21.4	0.6
7	TE - C - S- SXT - NA - K - E	4.8	0.6	TE - C-AMP-S -SXT- NA -E TE - C-AMP- SXT- NA -K- E TE -AMP-S - SXT- NA -K- E TE – C-S -SXT- NA -K- E TE -AMP-S - SXT- NA -K- E TE - C-AMP- CN- SXT-K- E TE - C-AMP-S - SXT-K- E TE - C-SXT- NA -K- E- CIP TE - C-AMP -SXT- NA - E- CIP	20.2	0.5

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 December 2020 doi:10.20944/preprints202012.0679.v1

				TE - C-AMP- NA - E- CIP- CL		
6	TE - C - AMP - S - SXT - E	4.8	0.5	TE - C-AMP- SXT- K- E TE - C-AMP-S - SXT- E TE - C-SXT- NA -E- CIP TE - C-AMP- SXT- NA – E TE -AMP-SXT- NA-K- E TE - C-AMP-NA - E- CIP TE -S - CN- SXT- K- E	17.2	0.5
5	S - SXT - NA - K - E TE - C - AMP - K - E	14.2	0.4	TE - C-AMP-SXT- E TE – C- S - SXT-E TE - C-AMP- K- E AMP-NA - E- CIP- CL TE -AMP-SXT- NA – E TE -AMP-SXT- K- E TE -AMP-S - SXT-E TE -AMP-NA - E- CIP C-AMP-SXT- K- E TE - AMP-S- NA - E	12.5	0.4
4	TE - C - AMP – E TE - C - SXT - E C - AMP - K – E C - AMP - SXT - E	42.8	0.3	TE - S - SXT- E TE - C-AM- E C-AMP-SXT-E TE -AMP-SXT-E TE -AMP-E- CL AMP-K- E- CL TE – C- SXT-E	6.5	0.3
3	SXT - C – E TE - C - E	(9.5	0.2	TE - AMP- E S - NA -E TE – C- E	3.5	0.2
2	N/A	N/A	N/A	AMP- E	0.5	0.1
1	E	19	0.08	N/A	0	0

TE, tetracycline; C, chloramphenicol; AMP, ampicillin; CL, cephalothin; S, streptomycin; CN, gentamicin; SXT, sulfamethoxazole/trimethoprim; NA, nalidixic acid; CIP, ciprofloxacin; E, erythromycin; K, kanamycin